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TOXICITY OF VARIOUS BENZENE DERIVATIVES TO INSECTS

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INTRODUCTION

The author in a previous paper (5)[1] pointed out the possibility of fumigating animals with nitrobenzene [2] to destroy their external parasites. In that work and in later experiments with nitrobenzene as many as 500 animals (cattle, sheep, hogs, chickens, dogs, cats, rats, and guinea pigs) have been fumigated, with only two cases of possible poisoning. One case was the fumigation of five chickens, which, through a misunderstanding of an assistant, were fumigated for 13 hours instead of 8, with the result that the chickens later died from paralysis of the central nervous system. The other instance was that of a young cat, which after one hour showed signs of uneasiness and was removed from the fumigation cage. As no symptoms of poisoning resulted in this case the cat may have been reacting to a strange environment rather than to the action of the poison.

Nevertheless, in view of the extreme toxicity of nitrobenzene as recorded in different works on toxicology, it was felt that it might be too poisonous for general use by inexperienced persons. For this reason a study of a series of benzene derivatives was undertaken with a view to determining their toxicity to insects; and from the result of this study it was hoped that one or more compounds might be found which would be quite toxic to insects while nontoxic to higher animals or plants. A study of the toxicity of the vapor of 28 benzene derivatives has been completed. A knowledge of the toxicity of the vapors of these compounds is valuable not alone for fumigation purposes but also as an

[1] Reference is made by number to "Literature cited," p. 380.

[2] In nomenclature the usage of American Chemical Society is followed.

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index of their worth as contact sprays, since Shafer (6) and, more recently, McIndoo (4) have shown that most contact sprays kill by the action of their vapor rather than by the plugging of the spiracles.

COMPOUNDS USED IN THE EXPERIMENTS

From the hydrocarbon benzene C_6H_6 a great many compounds may be derived by replacement of one or more of the hydrogen atoms by certain other elements or groups of elements. These compounds are designated "mono," "di," "tri," etc., derivatives, depending on the number of hydrogens which are substituted. The following mono-substitution compounds have been tested in this study:

Benzonitrile, C_6H_5CN
Chlorbenzene, C_6H_5Cl
Brombenzene, C_6H_5Br
Iodobenzene, C_6H_5I
Phenol, C_6H_5OH
Anilin, $C_6H_5NH_2$
Benzaldehyde, C_6H_5CHO
Nitrobenzene, $C_6H_5NO_2$
Toluene, $C_6H_5CH_3$

The following di-substitution products were employed:

Xylene, $C_6H_4(CH_3)_2$ (mixture of the three possible isomeres)
Para-dichlorbenzene, $C_6H_4Cl_2$
Para-dibrombenzene, $C_6H_4Br_2$
Ortho-chlorphenol, $C_6H_4OH\ Cl$
Ortho-nitrophenol, $C_6H_4OH\ NO_2$
Salicylic aldehyde, $C_6H_4O\ H\ CHO$

Besides these di-substitution compounds, several other derivatives were used, which may be considered di-substitution compounds of benzene or mono-substitutions of toluene. They were ortho- and para-bromtoluene ($C_6H_4CH_3Br$), ortho-, meta-, and para-cresol ($C_6H_4CH_3OH$), and ortho-nitrotoluene ($C_6H_4CH_3NO_2$). Inasmuch as different compounds are obtained by substitution in the methyl group of toluene rather than in the benzene ring of toluene, two such compounds were tested: Benzyl alcohol ($C_6H_5CH_2OH$) and benzoyl chlorid ($C_6H_5CO\ Cl$). Two derivatives of xylene were tried: Bromxylene ($C_6H_3(CH_3)_2Br$) and nitroxylene ($C_6H_3(CH_3)_2NO_2$).

The xylene used in the experiments was a mixture of ortho-, meta-, and para-xylene; hence, the bromxylene and nitroxylene were also mixed compounds.

In this series is shown a wide range of compounds very different in chemical composition. A few others were tested but not included, owing to their slight volatility.

METHODS OF EXPERIMENTATION

One-liter Florence flasks of pyrex glass, closed with rubber stoppers, were used as fumigation chambers. As rubber was found to absorb the vapor of the chemicals, the stopper was coated with lead foil. Measured quantities of the compound to be tested were placed on a piece of filter paper cut just as small as possible, the paper was suspended from the stopper inside of the flask, and the compound was allowed to evaporate. After several different insects were used in preliminary tests, the house fly (*Musca domestica* L.) was selected as being typical and easy to breed in large numbers. The flies were bred in the insectary and kept under natural conditions, thus avoiding irregular results due to the different ages and physical conditions of the wild flies. Five flies were put into each flask, the chemical introduced, and the flask tightly stoppered. When all the flies in the flask were apparently dead, they were removed to a vial and given 24 hours to revive. If none revived, the time during which the flies were exposed to the vapor was recorded. But, if the flies revived, the experiment was repeated. The average of 50 tests for a certain quantity of any chemical was found to be practically the same as the average of 5 tests; hence, in each case 5 tests were conducted. Controls showed that flies could live in a closed flask for 20 or more hours.

Since similar weights of the different chemicals do not contain the same number of molecules, and their toxicity could not, therefore, be accurately compared, it was decided to determine the toxicity in minutes for similar fractions of a gram-molecule of each chemical. Different quantities of each chemical were tested and curves plotted. As the quantity increased, it was found that each chemical had a point beyond which an increase would not give a reduction in the time required to kill. This is the point at which the air is saturated with the vapor, and differs for each chemical. As the quantity is decreased, a point is reached where the vapor is not of sufficient strength to kill. The plotted curves lie between these two points.

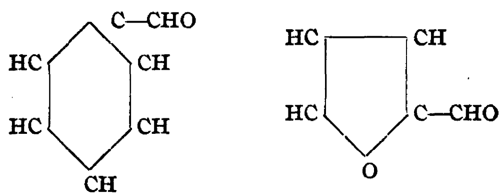
After the curves were plotted, it was found to be impossible to compare similar fractions of a gram-molecule; hence, the different fractions of a gram-molecule necessary to kill in a fixed time of 400 minutes were determined. A long period of time was selected as a more nearly correct index of toxicity. The fraction of the gram-molecule was determined by dividing the amount of the chemical necessary to kill in 400 minutes by the molecular weight of the substance. The sums given in the charts are the millionths of a gram-molecule necessary to kill five house flies in a 1-liter flask at a temperature of 70° F.

The liquid benzene compounds were measured by volume in blood-counting pipettes, and the weight of this volume was determined from the weight of 1 c. c. of the chemical. Weighed quantities of the solid benzene derivatives were dissolved in a known volume of benzene. A certain volume of this solution would contain a definite quantity of the benzene derivative. The measured volume was placed on the paper and blown for a moment to evaporate the solvent. The rapid evaporation of the solvent resulted in a lowering of the temperature, thus preventing appreciable evaporation of the compound to be tested.

RESULTS OF THE INVESTIGATION

Inasmuch as carbon bisulphid is in general use as a fumigant, its toxicity was determined for comparison with the toxicity of the benzene derivatives. Figure 1 shows curves based upon different amounts of certain of the chemicals and the time required to kill with such quantities. Owing to the extended curves of some of the substances a graphical representation was not feasible.

The data upon which the curves are based are given in Table I. Furfural is included for comparison with the aldehydes of benzene. Its relationship to benzenaldehyde is shown in the following formulas:



The results, given in millionths of a gram-molecule, are recorded in Table II and shown graphically in figure 2.

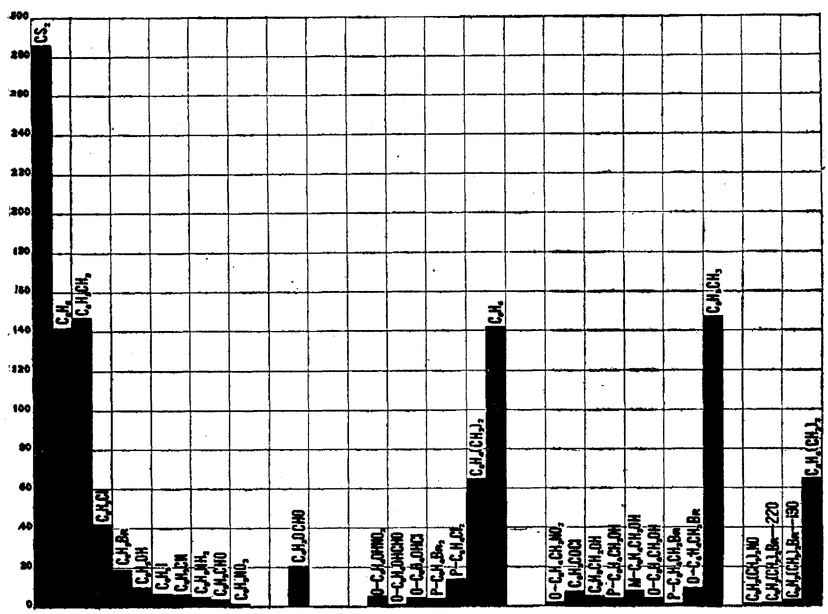


FIG. 2.—Graph showing the millionths of a gram-molecule necessary to produce the death of five house flies in a 1-liter flask at 70° F. in 400 minutes.

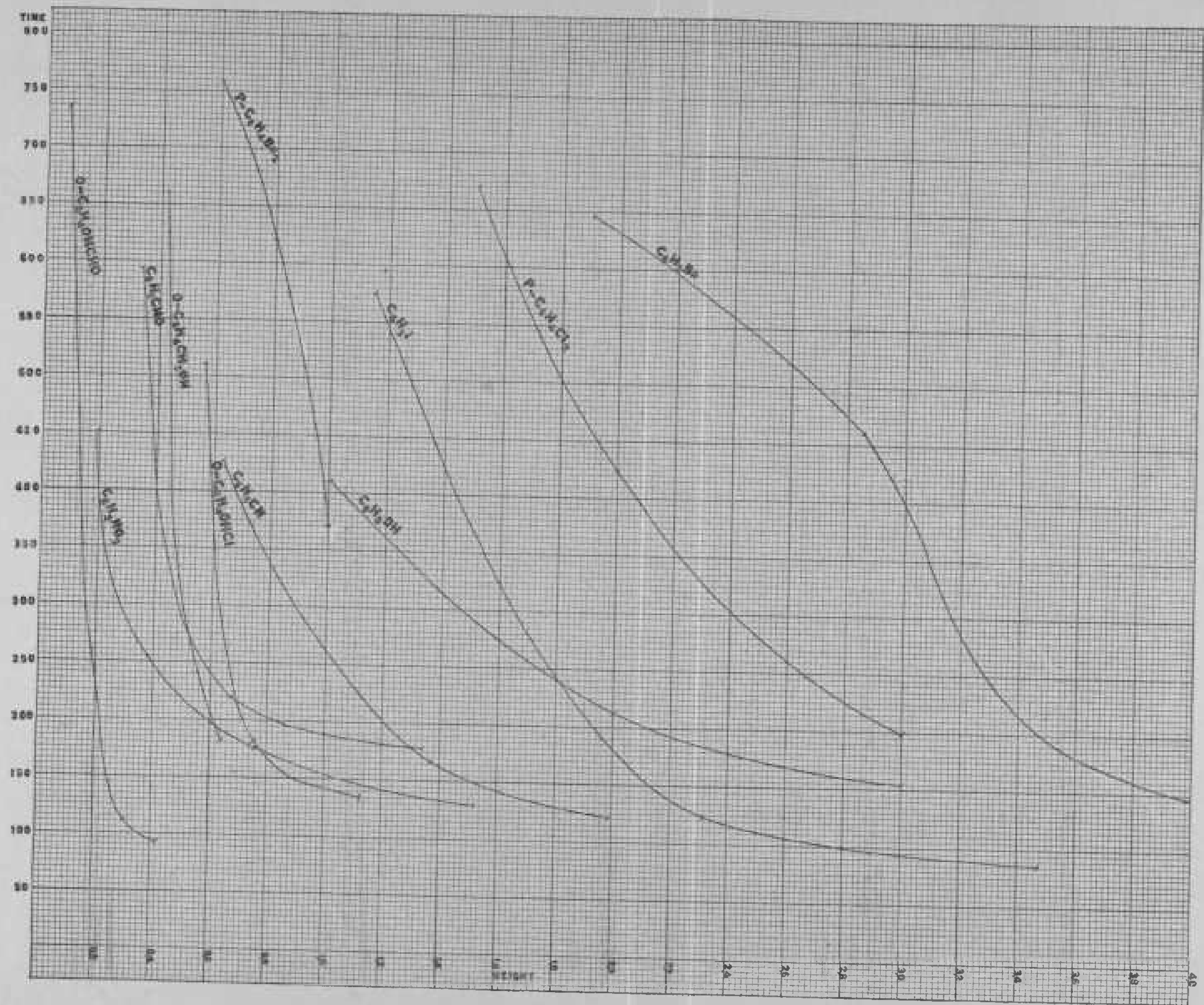


FIG. 1.—Curves showing periods of time required to kill with different quantities of the different benzene derivatives.

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TABLE I.—*Quantity of a chemical and time required to kill five house flies in a 1-liter flask at 70° F.*

Chemical.	Quantity of chemical required.	Time required (average of five tests).	Chemical.	Quantity of chemical required.	Time required (average of five tests).
	Gm.	*Minutes.*		*Gm.*	*Minutes.*
Carbon bisulphid	0.02179	400	Salicylic aldehyde	.00044	93
Benzene	.02028	185	Do	.00029	110
Do	.01521	349	Do	.00014	349
Do	.01015	427	Do	.00007	735
Toluene	.01548	258	Ortho-nitrophenol	.001	241
Do	.01289	498	Do	.0007	409
Do	.01032	600	Do	.0005	512
Chlorbenzene	.01265	85	Ortho-bromtoluene	.00265	159
Do	.00632	215	Do	.00177	341
Do	.00413	566	Do	.00132	610
Brombenzene	.00475	133	Para-bromtoluene	.001	318
Do	.0038	160	Do	.0005	350
Do	.0028	461	Do	.00025	351
Do	.0019	646	Do	.0001	544
Phenol	.003	155	Ortho-cresol	.00064	182
Do	.002	213	Do	.00048	333
Do	.001	411	Do	.00042	661
Iodobenzene	.00347	87	Meta-cresol	.00196	223
Do	.00231	123	Do	.00130	304
Do	.00115	576	Do	.00065	479
Benzonitrile	.00190	122	Para-cresol	.00193	249
Do	.00137	166	Do	.00129	319
Do	.00063	427	Do	.00064	360
Anilin	.00128	137	Do	.00032	435
Do	.00064	204	Benzoyl chlorid	.00257	130
Do	.00048	511	Do	.00164	273
Benzaldehyde	.00134	178	Do	.00082	486
Do	.00067	219	Ortho-nitrotoluene	.00058	281
Do	.00033	595	Do	.00036	293
Nitrobenzene	.00152	130	Do	.00029	450
Do	.00076	176	Bromxylene (B. P.		
Do	.00038	254	190°–250° C.)	.002	201
Do	.00019	451	Do	.001	292
Xylene	.01005	95	Do	.0005	487
Do	.00754	305	Bromxylene (B. P.		
Do	.00502	911	220°–250° C.)	.00087	269
Para-dichlorbenzene	.003	200	Do	.00043	356
Do	.002	424	Do	.00022	532
Do	.001	670	Nitroxylene	.00028	368
Para-dibrombenzene	.001	371	Do	.00014	581
Do	.0008	621	Furfural	.00297	155
Do	.0006	759	Do	.00223	273
Ortho-chlorphenol	.00112	131	Do	.00198	437
Do	.00075	181			
Do	.00056	512			

TABLE II.—*Quantity of a chemical necessary to kill five house flies in a 1-liter flask in an arbitrary time of 400 minutes*

Chemical.	Quantity of chemical required (in millionths of a gram-molecule).	Chemical.	Quantity of chemical required (in millionths of a gram-molecule).
Carbon bisulphid	286. 3	Salicylic aldehyde	1. 1
Benzene	142. 3	Ortho-nitrophenol	5. 6
Toluene	1475	Ortho-bromtoluene	9. 4
Chlorbenzene	42. 4	Para-bromtoluene	1. 2
Brombenzene	19. 2	Ortho-cresol	4. 2
Phenol	10. 8	Meta-cresol	7. 9
Iodobenzene	6. 6	Para-cresol	3. 9
Benzonitrile	6. 4	Benzyl alcohol	5. 3
Anilin	5. 3	Benzoyl chlorid	7. 8
Benzaldehyde	3. 7	Ortho-nitrotoluene	2. 1
Nitrobenzene	1. 8	Bromxylene (B. P. 190°–210° C.)	3. 5
Xylene	6. 4	Bromxylene (B. P. 220°–250° C.)	1. 9
Para-dichlorbenzene	14. 0	Nitroxylene	1. 7
Para-dibrombenzene	4. 1	Furfural	20. 8
Ortho-chlorphenol	4. 6		

DISCUSSION OF RESULTS

TOXICITY AND CHEMICAL COMPOSITION

By a glance at figure 2 it is noticed that all the benzene compounds used are more toxic than carbon bisulphid. The introduction of a methyl group into the benzene ring decreases its toxicity. This result agrees with the findings for higher animals of Winternitz and Hirschfelder (7) and further studies of Kline and Winternitz (3). The introduction of a halogen increases the toxicity similar to the results of Bechhold and Ehrlich (1), who found the introduction of a halogen increased the disinfection properties of phenol. This fact is true for insects whether the halogen is introduced in benzene, toluene, xylene, or phenol.

One might expect that, as toluene is less toxic than benzene, the halogen derivative of toluene would be less toxic than the similar derivative of benzene; but such does not seem to be the case. The iodin derivatives are more toxic than the corresponding bromin compounds, while both are more toxic than the corresponding chlorin derivatives. The disubstitution compounds of the halogens are more toxic than the monosubstitutions. The introduction of the cyanogen group does not increase the toxicity as much as might be supposed. The aldehyde group greatly increases the toxicity; in fact, salicylic aldehyde is the most toxic of all the compounds used in the experiments. From this result it would be expected that furfural would be much more poisonous than the results show it to be. Substitutions in the methyl group of toluene are less toxic than in the benzene ring. Para configurations seem to be more toxic than ortho configurations, while the only meta derivative tried was less toxic than either. Although certain relationships exist between chemical composition and toxicity, they are not as striking or as constant as might be expected.

BOILING POINTS AND TOXICITY

In working over the results, the author noticed a relationship between the boiling point of the chemical and its toxicity. As many of the compounds bore no guaranty of purity, the boiling points of several were determined and a curve plotted, showing the chemicals in order from the lowest boiling point to the highest. In comparison, a curve of toxicity of these compounds was plotted, as shown in figure 3. The curves show strikingly that the higher the boiling point the more toxic is the chemical. Exceptions are to be noted, which may be due to the rôle played by chemical composition in either raising or lowering the toxicity; but, in general, the curve is an increase of toxicity with an increase in the boiling point. Benzaldehyde shows a break in the curve, possibly owing to a specific action of the aldehyde. The low boiling point of furfural (96° C.) may account for its toxicity being less than would be expected from its chemical composition. Carbon bisulphid, having the lowest boiling point (47° C.), lower than any of the benzene derivatives tested, is likewise the least toxic of all the compounds.

The explanation of the relationship of boiling point to toxicity has not been ascertained. Whether the introduction of a certain element or group causes an increase in toxicity incidental to an increase in the boiling point or whether it is the relationship of boiling point to vapor pressure and volatility is not known.

BOILING POINT AND LIPOID SOLUBILITY

Another interesting observation is the relationship between boiling point and lipoid solubility. To test the lipoid solubility of the compounds, cephalin was extracted from the brain of an ox by Hirschfelder's method (2).

Ox brain was covered with three volumes of alcohol, shaken up two or three times, and the excess of alcohol then poured off and squeezed out gently through linen, care being taken to avoid great force in wringing out the alcohol, as this tends to break up the brain tissue into very finely divided particles which pass through the filter. The residue is then covered with three volumes of ether, shaken vigorously, and filtered first through cotton and then through filter paper. The clear filtrate thus obtained is evaporated to dryness over a water bath and a yellow residue remains.

The cephalin so prepared was placed in capsule heads of 0.08 c. c. capacity and introduced into 1 c. c. of the chemical to be tested. It was found that benzene boiling at 78.5° C., toluene at 107.5° C., and xylene at 130° C. dissolved several capsules of cephalin until it finally

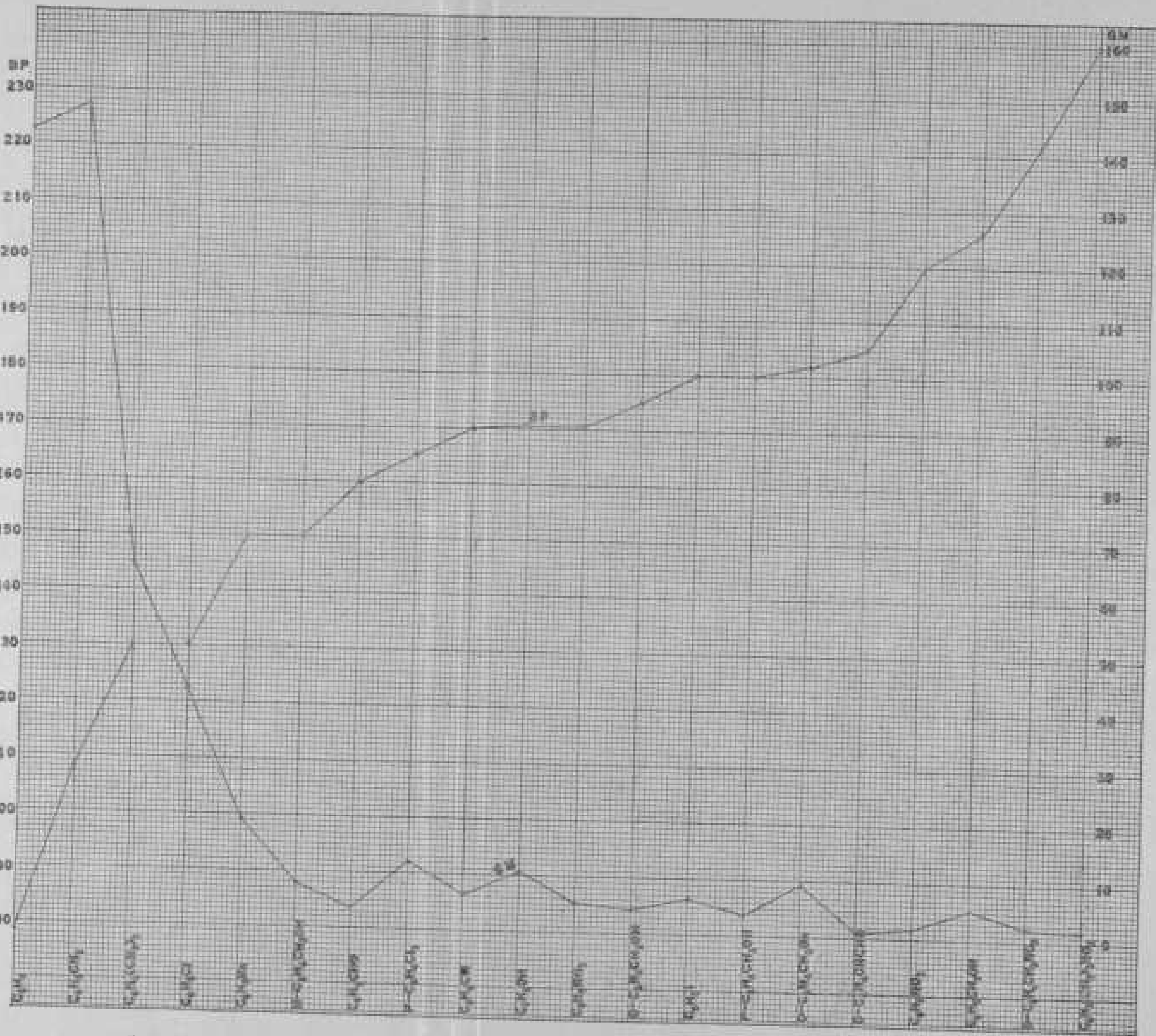

FIG. 3.—Curves showing the relationship between boiling point (BP) of the compound and its toxicity expressed in millionths of a gram-molecule (GM).

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became thick and pastelike. By placing cephalin under a small bell jar, the air of which remained saturated with benzene, it absorbed benzene from the air until finally a liquid mass was produced. The same was true for toluene and xylene. This shows that cephalin and either benzene, toluene, or xylene are miscible in all proportions. On the other hand, brombenzene, with a boiling point of 150° C., dissolved but one capsule in 1 c. c. Benzaldehyde (165° C.) slowly penetrated the cephalin, but dissolved but little of it; while anilin (170.5° C.) salicylic aldehyde (185° C.), nitrobenzene (200° C.), and nitroxylene (240° C.) did not penetrate the cephalin and dissolved but very slight traces of it. Five c. c. of nitrobenzene, evaporated to dryness, left a very slight greasy mark on the evaporating dish. An effort was made to extract cephalin from the brain tissue with nitrobenzene without success. Lanolin also is practically insoluble in nitrobenzene. One c. c. of benzene containing 0.16 c. c. of cephalin was poured into 10 c. c. of nitrobenzene and the mixture blown with an electric fan until the benzene was evaporated, resulting in the cephalin's being thrown out of solution. From these results it appears that compounds with high boiling points are poor lipoid solvents, but are the most toxic to insects. These experiments would indicate that an increase in lipoid solubility as determined by the above method causes a decrease in toxicity in the chemicals used. Further work is now in progress to determine whether a similar relationship exists between the boiling point, lipoid solubility, and toxicity of a wider range of chemicals from the alipathic series and the terpenes.

TOXICITY OF BENZENE DERIVATIVES TO OTHER INSECTS

The toxicity of the benzene derivatives was found to be similar for other insects, and although this work has not been completed, one point may be noted. A comparison of the bluebottle fly (*Lucilia sericata* Mg.) with the house fly (*Musca domestica* L.) shows that house flies die more quickly from compounds with a low boiling point than bluebottle flies, while compounds with a high boiling point are more toxic to the bluebottle flies than to the house fly. Similarly, the cockroach (*Blatella germanica* Linn.) succumbs less readily than the potato beetle (*Leptinotarsa decemlineata* Say) to low boiling compounds and more readily to high boiling compounds. This relationship may be due to morphological differences in the insects, possibly the diameter of the spiracles or trachea.

CONCLUSIONS

Although no effort has yet been made to apply the results, certain possibilities are apparent. Even if the compounds with low boiling points are less toxic than those with high boiling points, inasmuch as more of such compounds may be evaporated before saturation is reached, better results may be obtained. This is shown in figure 4, which gives the maximum amount (in pounds) that will evaporate in 1,000 cubic feet of

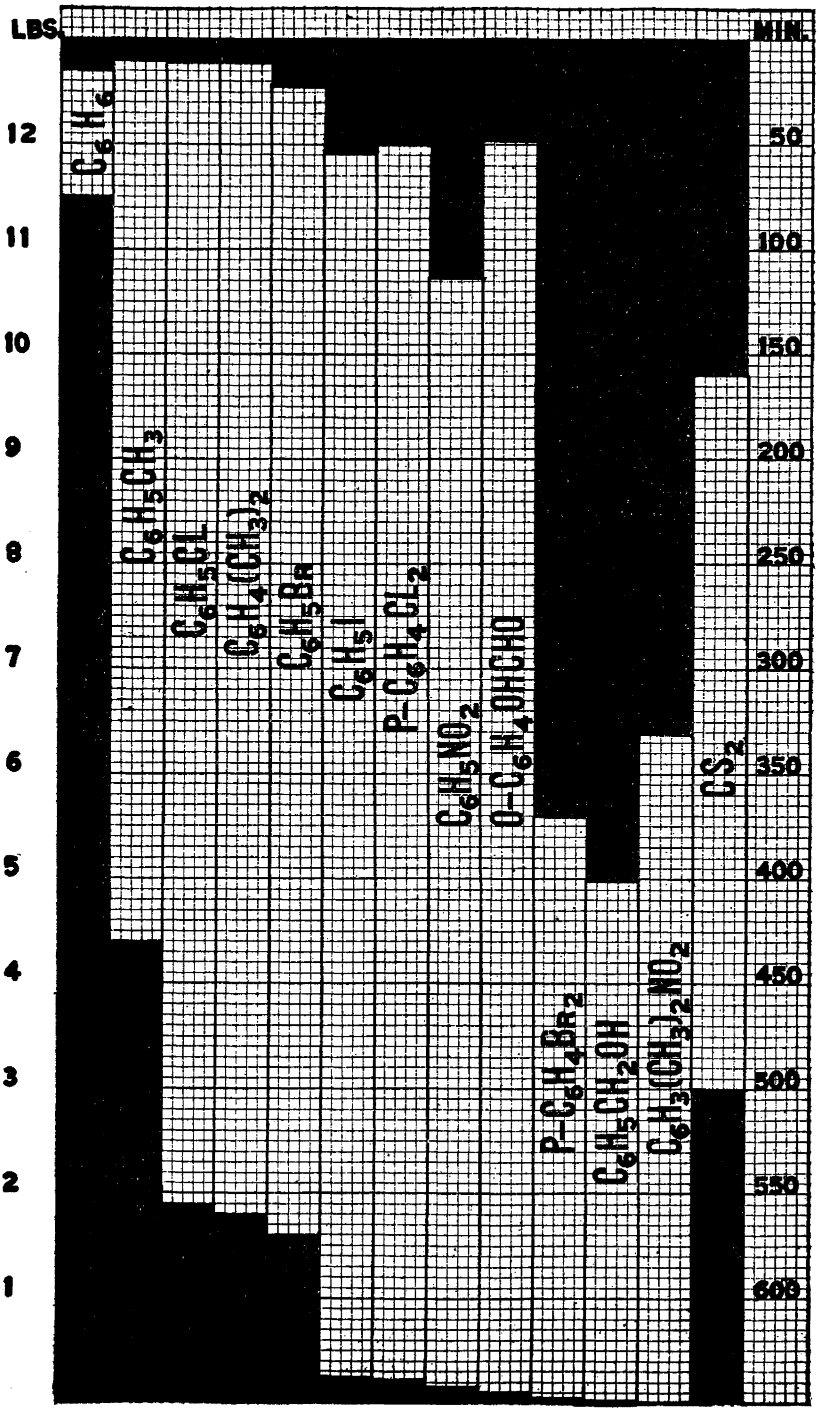

FIG. 4.—Graph showing the quantity of the benzene derivatives necessary to saturate 1,000 cubic feet of space at 70° F. and the time required by such quantity to kill house flies. Carbon bisulphid at the standard rate is given for comparison.

space at 70° F., and the time required for such quantity to kill house flies. Carbon bisulphid at the rate of 3 pounds to 1,000 cubic feet is compared with the benzene derivatives. As a low-boiling compound will penetrate grain better than a high-boiling compound, the possibilities of xylene, chlorbenzene, and brombenzene are at once apparent. Tests of the value of these compounds in the fumigation of grain have not been made. Inasmuch as the vapor of many of the benzene compounds is explosive when mixed with air, one must observe certain precautions, although in general they are far less explosive than carbon bisulphid.

For the fumigation of animals a compound with a high boiling point is needed in order that relatively little of the material shall be in the air to be taken in by the animal or to irritate the eyes or nose. In this respect salicylic aldehyde is probably the best. The cost of this chemical is prohibitive for general fumigation; but, inasmuch as higher animals readily oxidize it to salicylic acid, which is very slightly poisonous, this compound might be used for the internal fumigation of horses to destroy bots as carbon bisulphid is now used. As previously stated, it has been decided to try out a large series of chemicals before selecting the best compounds for tests as to their practicable possibilities.

SUMMARY

Data are presented showing the toxicity of certain organic compounds, mainly from the aromatic series, to insects, particularly the house fly, and certain general relationships are indicated.

(1) All the benzene derivatives tested proved to be more toxic to insects, molecule for molecule, than carbon bisulphid.

(2) Physical characters, such as boiling point and vapor pressure, have more influence on the toxicity than chemical composition.

(3) Up to 250° C. the higher the boiling point the more toxic the compound to insects. Beyond 250° C. the compound is usually so slightly volatile that not enough of the chemical will evaporate to be effective.

(4) Lipoids are very soluble in compounds with low boiling points and but slightly soluble in compounds with high boiling points.

(5) Compounds with low boiling points, although less toxic, owing to their great volatility, may give better results than compounds with high boiling points, particularly in the fumigation of grain.

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